Activation of 5-HT_{1A} Receptors in the Hypothalamic Paraventricular Nuclei Negatively Regulates Cytochrome P450 Expression and Activity in Rat Liver

Ewa Bromek,¹ Marta Rysz,¹ Anna Haduch, Jacek Wójcikowski, and Władysława A. Daniel

Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

Received November 21, 2017; accepted March 14, 2018

ABSTRACT

Our recent work suggested a negative effect for the serotonergic innervation of the paraventricular nuclei (PVN) of the hypothalamus on growth hormone secretion and growth hormone-dependent expression of CYP2C11. The aim of our present research was to determine the effect of the activation of the 5-hydroxytryptamine [(5-HT) serotonin] 5-HT₁ or 5-HT₂ receptors in the PVN on the expression and activity of cytochrome P450 in male rat liver. The serotonergic agonists 5-carboxyamidotryptamine [(5-CT), a 5-HT₁ receptor-type agonist], 8-hydroxy-2-(di-*n*-propylamino)-tetralin [(8-OH-DPAT), a 5-HT_{1A} receptor agonist], sumatriptan (a 5-HT_{1B/D} receptor agonist), and 2,5-dimethoxy-4-iodoamphetamine [(DOI), a 5-HT_{2A/C} receptor agonist] were individually injected into the PVN. The liver cytochrome P450 activity and expression and the levels of serum and pituitary and hypothalamic hormones were measured. 5-CT and 8-OH-DPAT

significantly decreased the activity and expression of CYP2C11 at both the mRNA and protein levels, which was accompanied by an increase in pituitary and hypothalamic somatostatin levels and a decrease in the serum growth hormone concentration. The expression of CYP3A1/23 also decreased. The serum corticosterone concentration declined after the injection of 8-OH-DPAT. The obtained results indicated that 5-HT_{1A} but not the 5-HT_{1B/D} or 5-HT₂ receptors in the PVN are engaged in the negative neuroendocrine regulation of cytochrome P450 via the stimulation of hypothalamic somatostatin secretion and in the decreases in the serum growth hormone and corticosterone concentrations. Since the affected enzymes metabolize steroids and drugs and 5-HT_{1A} receptors are engaged in the action of psychotropic drugs, the results obtained may be of both physiologic and pharmacological meaning.

Introduction

The physiologic regulation of the expression of liver cytochrome P450 (P450) is governed by hormones such as growth hormone (GH), glucocorticoids, thyroid, and sex hormones. By acting on membrane, cytoplasmic, or nuclear receptors, they regulate the transcription of genes coding for P450 isoforms, affecting the expression and activity of particular hormone-dependent P450 isoforms (Waxman and O'Connor, 2006; Waxman and Holloway, 2009; Monostory et al., 2009; Dvorak and Pavsek, 2010; Brtko and Dvorak, 2011; Monostory and Dvorak, 2011; Wójcikowski and Daniel, 2011).

Our earlier studies showed the important role of brain monoaminergic systems, such as the dopaminergic (Wójcikowski et al., 2007, 2008; Wójcikowski and Daniel, 2009), noradrenergic (Sadakierska-Chudy et al., 2013; Kot et al., 2015), and serotonergic (Rysz et al., 2015, 2016a) systems in the physiologic neuroendocrine regulation of P450 expression and activity in the liver. They indicated opposite effects of the catecholaminergic and serotonergic systems on the regulation of the

expression and activity of CYP2C11, CYP3A, CYP2B, or CYP2A isoforms (positive or negative, respectively), but positive effects of both those systems on CYP1A expression and activity. Moreover, the results obtained to date showed the important but reverse effects of monoaminergic innervations (both catecholaminergic and serotonergic) of the hypothalamic paraventricular nuclei (PVN) and arcuate (ARC) nuclei on the regulation of CYP2C11 and CYP3A isoforms, which were negative in the case of PVN and positive in the case of ARC and involved corresponding changes in GH secretion (Wójcikowski and Daniel, 2009; Bromek et al., 2013; Rysz et al., 2016b).

The main role in the central neuroendocrine regulation of liver P450 is played by the hypothalamus, which contains the PVN-producing corticotropin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH), and somatostatin (SST), and the ARC, which produces the GH-releasing hormone. These hypothalamic hormones act differently on the secretion of anterior pituitary gland hormones engaged in the regulation of P450 expression. Both the PVN and ARC are innervated by serotonergic projections originating from the frontal raphe serotonin-containing neuronal groups, mainly from the dorsal and median raphe nuclei (Sawchenko et al., 1983; Gruber et al., 1987; Willoughby and Blessing, 1987; Larsen et al., 1996). Serotonin [5-hydroxytryptamine (5-HT)] released in the PVN or ARC stimulates serotonergic receptors located therein and thus may affect the synthesis and release of liberating

This study was financially supported by the National Science Centre, Kraków, Poland [Grant OPUS 6 No. 4 2013/11/B/NZ7/04897] and by statutory funds from the Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland.

¹E.B. and M.R. contributed equally to this work.

<https://doi.org/10.1124/dmd.117.079632>

ABBREVIATIONS: ACTH, adrenocorticotrophic hormone; ARC, arcuate nucleus; CRH, corticotropin-releasing hormone; CRT, corticosterone; 5-CT, 5-carboxyamidotryptamine; DOI, 2,5-dimethoxy-4-iodoamphetamine; ELISA, enzyme-linked immunosorbent assay; GH, growth hormone; 5-HT, 5-hydroxytryptamine (serotonin); IL, interleukin; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)-tetralin; P450, cytochrome P450; PCR, polymerase chain reaction; PVN, paraventricular nuclei; SST, somatostatin; T₃, triiodothyronine; T₄, thyroxine; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone.

(CRH, TRH, or GH-releasing hormone) or inhibiting (SST) hormones. These hormones, in turn, affect pituitary hormone secretion.

It is assumed that the stimulating effect of brain serotonin on GH release can be initiated by either GH-releasing hormone release from the ARC via 5-HT₂ receptors (Murakami et al., 1986; Willoughby et al., 1987; Katz et al., 1996) or the inhibition of SST release from the PVN. The latter effect likely proceeds via 5-HT₁ receptors, as suggested in humans and dogs (Mota et al., 1995; Seletti et al., 1995; Valverde et al., 2000; Pitchot et al., 2004). The peripheral or intracerebral administration of selective serotonergic agonists indicated that the stimulatory effect of serotonin on the synthesis and release of CRH from the PVN is mediated by 5-HT_{1A} and 5-HT_{2A/2C} receptors (Jørgensen, 2007). The stimulation of thyroid hormone release may occur via the inhibition of SST secretion, while the inhibitory action seems to stem from the suppression of TRH release from the hypothalamic PVN by serotonin (Tuomisto and Männistö, 1985; Toivonen et al., 1990).

Our previous study carried out after overall lesion or activation of the brain serotonergic system indicated the engagement of that system in the central neuroendocrine regulation of P450 expression in the liver (Rysz et al., 2015, 2016a). The follow-up study performed after damage to the serotonergic innervation of the hypothalamic nuclei PVN or ARC showed the important role of these brain structures in the aforementioned enzyme regulation (Rysz et al., 2016b). Damage to the PVN led to an increase in serum GH and testosterone concentrations, subsequently leading to an increase in the expression (mRNA and protein level) and activity of CYP2C11, the main P450 isoform in male rat liver (Rysz et al., 2016b). Since both 5-HT₁ and 5-HT₂ receptor types are present in the PVN (Zhang et al., 2004), the aim of our present research was to identify the 5-HT₁ or 5-HT₂ receptor types/subtypes located in the PVN that are involved in P450 expression in the liver. To this end, we administered different selective 5-HT receptor agonists in pharmacologically active doses into the PVN and then examined the levels of serum, pituitary and hypothalamic hormones, and liver P450 function.

Materials and Methods

Animals. Male Wistar Han rats (Charles River Laboratories, Sulzfeld, Germany), weighing 280–300 g, were housed under standard laboratory conditions (12:12 hour light/dark cycle; temperature of 22°C ± 2°C; room humidity of 55% ± 5%). The animals had free access to food and tap water; however, 18 hours before decapitation, they were deprived of food. The experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (2011), and with approval of the Local Bioethics Commission at the Institute of Pharmacology of the Polish Academy of Sciences (Kraków).

Drugs and Chemicals. The following serotonergic receptor agonists were used: 5-carboxyamidotryptamine (5-CT) maleate, 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT) hydrobromide, and sumatriptan succinate, which were obtained from Sigma (St. Louis, MO); and 2,5-dimethoxy-4-iodoamphetamine (DOI) hydrochloride, which was obtained from Tocris (Bristol, United Kingdom). Caffeine, its metabolites, and RNA-free water were purchased from Sigma. Testosterone and its metabolites were provided by Steraloids (Newport, KY). The polyclonal primary anti-rat CYP2C11 antibody (obtained from Abcam, Cambridge, United Kingdom), anti-rat CYP3A1/23 and CYP3A2 antibodies (obtained from Millipore, Temecula, CA), and polyclonal anti-rat β -actin antibody (obtained from Santa Cruz, Dallas, TX) were applied. The chemiluminescence reagents LumiGlo kit was obtained from KPL (Gaithersburg, MD). The following enzyme-linked immunosorbent assay (ELISA) kits were used: GH and corticosterone (CRT) (obtained from DRG MedTek, Warsaw, Poland); triiodothyronine (T₃) and thyroxine (T₄) (obtained from Cloud-Clone Corp., Katy, Texas); CRH, adrenocorticotrophic hormone (ACTH), TRH, and thyroid-stimulating hormone (TSH) (obtained from Elabscience, Bethesda, MD); pituitary SST (obtained from MyBiosource, San Diego, CA); and interleukin (IL)-2 and IL-6 (obtained from R&D Systems, Minneapolis, MN). A mirVana kit, TaqMan assays, and the TaqMan Gene Expression Master Mix (obtained from

Life Technologies, Carlsbad, CA), and a Transcriptor High-Fidelity cDNA synthesis kit (obtained from Roche Diagnostics, Indianapolis, IN) were used. Ketamine hydrochloride and xylazine hydrochloride were purchased from Biowet (Puławy, Poland).

Surgery and Intracerebral Injection of the Serotonergic Receptor Agonists 5-CT, 8-OH-DPAT, Sumatriptan, or DOI. The rats were anesthetized with ketamine HCl and xylazine HCl (65 and 5 mg/kg i.p., respectively) and were placed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). Stainless-steel guide cannulas were implanted bilaterally into the PVN of the hypothalamus. The following coordinates were used: anterior-posterior from the bregma, −1.6; lateral, ± 0.3; and ventral from the surface of the dura, −5.2 (Paxinos and Watson, 2007). Intracerebral injections were made through an inner cannula (ventral, −7.2) 7 days after implanting the guide cannula. The serotonergic agonists 5-CT (a general 5-HT₁ receptor agonist), 8-OH-DPAT (a 5-HT_{1A} receptor agonist), sumatriptan (a 5-HT_{1B/D} receptor agonist), and DOI (a 5-HT_{2A/2C} receptor agonist) were dissolved in a 0.9% NaCl solution and injected into the PVN at a pure substance concentration of 10 μ g/ μ l (0.5 μ l infused into one side at a rate of 0.5 μ l/min for 5-CT, 8-OH-DPAT, and sumatriptan or 6 μ g/ μ l (0.5 μ l infused into one side at a rate of 0.5 μ l/min) for DOI. The agonists were injected as a single dose or for 5 days once a day. Control animals (sham-operated rats, $n = 10$) were subjected to the same procedure as the 5-HT receptor agonist-treated group ($n = 10$), except that they received 0.9% NaCl (0.5 μ l) instead of an agonist. The 0.9% NaCl or agonist solution was administered into the PVN using a Hamilton syringe (0.5 μ l/min), which was left in place for 5 minutes after injection. The rats were decapitated, and their brains were rapidly removed. The upper and lower parts of the middle hypothalamus (containing the PVN or ARC, respectively) and the pituitary gland were dissected. The placement of the needle was histologically verified as reported previously (Rysz et al., 2016b).

Collection of Brain, Pituitary, Liver, and Serum Samples. At 30 minutes after a single injection or at 3 hours after the last injection from the 5-day administration of a serotonergic agonist, the rats were decapitated. The middle hypothalamus, pituitaries, and livers were removed and stored at −80°C until they were further analyzed. The blood serum was separated by centrifugation and stored at −80°C. Liver microsomes were prepared from individual animals by differential centrifugation as described previously (Kot and Daniel, 2011).

Determination of Cytochrome P450 Isoform Activities in Liver Microsomes. The activity of CYP1A was examined by measuring the rate of caffeine metabolism (C-8-hydroxylation and 3-*N*-demethylation), as described previously (Kot and Daniel, 2008). The activities of CYP2A, CYP2B, CYP2C11, and CYP3A were evaluated by measuring the rate of testosterone 7 α -, 16 β -, 2 α - and 16 α -, and 6 β -hydroxylation, respectively, as described previously (Haduch et al., 2006, 2008; Wójcikowski et al., 2013).

An Analysis of the Cytochrome P450 Proteins in Liver Microsomes. The protein levels of CYP2C11, CYP3A1/23, and CYP3A2 in the liver microsomes (10 μ g of proteins) of control and serotonergic agonist-treated rats were estimated by western immunoblot analysis (a semiquantitative method), as previously described (Rysz et al. 2016a,b). Polyclonal rabbit anti-rat CYP2C11, CYP3A1/23, or CYP3A2 antibodies and a secondary antibody (a species-specific horseradish peroxidase-conjugated anti-IgG) were used. Rat cDNA-expressed CYP2C11 (5 μ g), CYP3A1/23 (1 μ g), and CYP3A2 (1 μ g) (Supersomes, Gentest, Woburn, MA) were used as standards. The intensity of the bands on a nitrocellulose membrane was quantified with a luminescent image analyzer and the data were normalized to protein loading based on the β -actin levels (Rysz et al. 2016a,b).

An Analysis of the Serum, Pituitary and Hypothalamic Hormones, and Serum Cytokines. Hormonal effects were measured 30 minutes after a single injection and 3 hours after the repeated administration of one of the serotonergic agonists. The levels of serum and pituitary or hypothalamic (PVN) hormones were measured using the following ELISA kits: GH kits (DRG, MedTek); CRT, T₃, and T₄ kits (Cloud-Clone Corp); CRH, ACTH, TRH, and TSH kits (Elabscience). The pituitary and hypothalamic (ARC) SST levels were measured using an ELISA kit from MyBiosource. The serum concentrations of cytokines were analyzed using the IL-2 and IL-6 kits (R&D Systems). Absorbance was measured using a microplate reader (Rysz et al. 2016a,b).

Isolation of Liver RNA and Quantitative Real-Time Polymerase Chain Reaction (PCR) Measurements. RNA was isolated from the liver, and quantitative real-time PCR was performed as described previously (Rysz et al., 2016b). Briefly, frozen liver tissue was homogenized, total RNA was extracted

Fig. 1. The effect of the repeated injection of the 5-HT₁ receptor agonist 5-CT (A) and the 5-HT₂ receptor agonist DOI (B) into the PVN (5 or 3 μ g/site, respectively) on different P450 isoform activities, measured as the rates of P450 isoform-specific reactions in rat liver microsomes: caffeine 8-hydroxylation and 3-N-demethylation (CYP1A) and testosterone 7 α - (CYP2A), 16 β - (CYP2B), 2 α - and 16 α - (CYP2C11), and 6 β - (CYP3A) hydroxylation. All values are the mean $\pm$ S.E.M. ($n = 8-10$). Statistical significance was assessed by one-way analysis of variance followed by Duncan's test and is indicated as $*P < 0.05$ compared with the control. The control values (picomoles per milligram protein per minute) are as follows: (A and B) 16.8 $\pm$ 2.3 (caffeine 8-hydroxylation); 3.5 $\pm$ 0.4 (caffeine 3-N-demethylation); and 86.4 $\pm$ 10.2, 22.1 $\pm$ 1.5, 540.7 $\pm$ 73.9, 484.5 $\pm$ 71.1, and 535.9 $\pm$ 50.9 (testosterone 7 α -, 16 β -, 2 α -, 16 α -, and 6 β -hydroxylation, respectively).

using a mirVana isolation kit, and then the first-strand cDNA products were generated using a Transcriptor High Fidelity cDNA Synthesis Kit. The expression of the genes encoding the P450 isoforms CYP2C11, CYP3A1/23, and CYP3A2 (*CYP2C11*, *CYP3A1/23*, and *CYP3A2*) and the reference genes *HPRT1* and *GAPDH* were detected by real-time PCR using commercially available TaqMan Gene Expression Master Mix and species-specific TaqMan-type probes and primers. The gene names for the tested P450 genes and two reference genes with identification numbers of the TaqMan primers used in the study are as follows: *CYP2C11* (Rn01502203_m1), *CYP3A1/23* (Rn03062228_m1), *CYP3A2* (Rn00756461_m1), *HPRT1* (Rn01527840_m1), and *GAPDH* (Rn01462662_g1). Real-time PCR runs were performed using the Bio-Rad CFX96 PCR system (Bio-Rad, Hercules, CA) as described previously (Rysz et al., 2016b). The PCR reactions of *CYP2C11*, *CYP3A1/23*, and *CYP3A2* and reference genes were run in duplicate. The level of P450 transcripts was normalized to *HPRT1* and *GAPDH* expression, and the relative quantification was obtained by a comparative $2^{-\Delta\Delta C_t}$ method. The amount of the investigated transcript was expressed as the fold change in the expression level relative to the calibrator (i.e., the average ΔC_t of the control group).

Statistical Analysis of the Data. The obtained values are shown as the mean $\pm$ S.E.M. of 8–10 rats. Changes in the levels of hormones, interleukins, P450 activity, protein, and mRNA levels were evaluated for statistical significance using one-way

Fig. 2. The effect of the repeated injection of the 5-HT_{1A} receptor agonist 8-OHDPAT (A) and the 5-HT_{1B/D} receptor agonist sumatriptan (B) into the PVN (5 μ g/site) on different P450 isoform activities, measured as the rates of P450 isoform-specific reactions in rat liver microsomes: caffeine 3-N-demethylation and 8-hydroxylation (CYP1A) and testosterone 7 α - (CYP2A), 16 β - (CYP2B), 2 α - and 16 α - (CYP2C11), and 6 β - (CYP3A) hydroxylation. All values are the mean $\pm$ S.E.M. ($n = 8-10$). Statistical significance was assessed by Student's *t*-test and is indicated as $*P < 0.05$, compared with the control. The control values (picomoles per milligram protein per minute) are as follows: (A) 31.9 $\pm$ 3.3 (caffeine 8-hydroxylation); 6.2 $\pm$ 0.6 (caffeine 3-N-demethylation); and 85.6 $\pm$ 9.7, 17.5 $\pm$ 1.7, 294.6 $\pm$ 23.8, 233.4 $\pm$ 23.1, and 611.9 $\pm$ 49.2 (testosterone 7 α -, 16 β -, 2 α -, 16 α -, and 6 β -hydroxylation, respectively); (B) 19.2 $\pm$ 2.1 (caffeine 8-hydroxylation); 6.4 $\pm$ 0.4 (caffeine 3-N-demethylation); and 81.7 $\pm$ 16.3, 10.4 $\pm$ 2.3, 220.2 $\pm$ 70.0, 417.3 $\pm$ 33.2, and 366.6 $\pm$ 98.4 (testosterone 7 α -, 16 β -, 2 α -, 16 α -, and 6 β -hydroxylation, respectively).

analysis of variance followed by Duncan's post hoc test, or by two-tailed Student's *t* test. The results were considered as statistically significant when $P < 0.05$.

Results

Studies into liver P450 activity and expression were conducted after the administration of pharmacological doses of the selected serotonergic agonists into the PVN. The effective doses of the agonists were chosen on the basis of the literature data showing characteristic biochemical and/or behavioral effects of the intracerebrally administered 5-CT (Mamede Rosa and Prado, 1997; Fletcher and Korth, 1999), DOI (Willins and Meltzer, 1997; Fletcher et al., 2002; Currie et al., 2010; de Paula et al., 2012), 8-OH-DPAT (Fletcher et al., 2002; de Souza Villa et al., 2008; Clissold et al., 2013), or sumatriptan (Piñeyro and Blier, 1996; Hopwood and Stamford, 2001; Johnson et al., 2001; Guo and Rainnie, 2010).

Fig. 3. The effect of the repeated injection of the 5-HT_{1A} receptor agonist 5-CT (A and B) and the 5-HT_{1A} receptor agonist 8-OHDPAT (C and D) into the PVN (5 μ g/side) on the protein levels of CYP2C11, CYP3A1/23, and CYP3A2 isoforms in rat liver microsomes. Microsomal proteins (10 μ g) were subjected to western immunoblot analysis. The presented results are typical of four (for the control and for 5-CT or 8-OHDPAT) separate rats per treatment (A and C). The data are expressed as the mean $\pm$ S.E.M. ($n = 8$) (B and D). Statistical significance was assessed by Student's *t*-test and is indicated as * $P < 0.05$ and ** $P < 0.01$, compared with the control.

The Effect of the Injection of 5-HT₁ and 5-HT₂ Receptor Agonists into the PVN on Cytochrome P450 Activity in the Liver.

In the liver microsomes of rats receiving an injection of 5-CT (a nonselective 5-HT₁ receptor agonist) into the PVN (5 μ g/side) for 5 days, the activity of CYP2C11 was measured as the rate of the 2 α - and 16 α -hydroxylation of testosterone, which was decreased (to 71% and 78% of the control, respectively) at 3 hours after the last injection (Fig. 1A). The activity of CYP2A evaluated as the rate of the 7 α -hydroxylation of testosterone significantly increased (to 126% of the control). The activities of CYP2B and 3A (assessed as the rate of the 16 β - or 6 β -hydroxylation of testosterone, respectively), as well as CYP1A (measured as the rate of caffeine C-8-hydroxylation and 3-*N*-demethylation), did not significantly change. The repeated injection of DOI (a nonselective 5-HT₂ receptor agonist) into the PVN (3 μ g/side) also elevated the activity of CYP2A (to 123% of the control), but it did not affect the activity of CYP2C11 or other isoenzyme subfamilies studied (Fig. 1B).

The 5-day injection of 8-OH-DPAT (a selective 5-HT_{1A} receptor agonist) into the PVN (5 μ g/side) significantly diminished the activity of CYP2C11 (which performs the 2 α - and 16 α -hydroxylation of testosterone) at 3 hours after the last injection, but it did not significantly affect the activities of CYP1A, 2A, 2B, or 3A (Fig. 2A). In contrast, the repeated injection of sumatriptan (a 5-HT_{1B/D} receptor agonist) into the PVN (5 μ g/side) did not influence the activity of CYP2C11 or other isoenzyme subfamilies tested (Fig. 2B). However, both 5-CT and 8-OH-DPAT tended

to decrease the activity of CYP3A measured as the 6 β -hydroxylation of testosterone (Figs. 1A and 2A).

The Effect of the Injection of 5-HT₁ Receptor Agonists into the PVN on Cytochrome P450 Expression in the Liver. Searching for the molecular mechanisms of the 5-HT₁/5-HT_{1A} receptor agonist-evoked decreases in the activity of CYP2C11, the enzyme expression was investigated in liver tissue after the repeated injection of the agonists into the PVN. The observed decreases in the activity of CYP2C11 correlated positively with the enzyme expression level observed at 3 hours after the last injection of the 5-HT₁ receptor agonist 5-CT or the 5-HT_{1A} receptor agonist 8-OH-DPAT (Fig. 3; Table 1). 5-CT significantly reduced the level of the CYP2C11 protein to 54% of the control (Fig. 3A), while 8-OH-DPAT diminished the enzyme protein level to 73% of the control (Fig. 3B). Accordingly, the level of *CYP2C11* mRNA significantly decreased after the injection of 5-CT or 8-OH-DPAT (Table 1). At the same time, the CYP3A1/23 protein level decreased (to 77% and 62.5% of the control, respectively), and the level of *CYP3A1/23* mRNA diminished in parallel after the repeated injection of 5-CT or 8-OH-DPAT (Fig. 3; Table 1).

The Effect of the Injection of 5-HT₁ Receptor Agonists into the PVN on the Levels of Serum, Pituitary and Hypothalamic Hormones, and Serum Cytokines. In searching for the central neuroendocrine mechanisms of the 5-HT₁/5-HT_{1A} receptor agonist-evoked decreases in *CYP2C11* and *CYP3A1* expression, the concentrations of the hormones engaged in the regulation of liver P450 were

TABLE 1

The effect of the repeated injection of the 5-HT₁ receptor agonist 5-CT and the 5-HT_{1A} receptor agonist 8-OH-DPAT into the PVN (5 μ g/side) on the mRNA expression level of the *CYP2C11*, *CYP3A1/23*, and *CYP3A2* genes in rat liver

The results are expressed as the fold change in relation to two housekeeping genes, *HPRT1* and *GAPDH*. All of the values are the mean fold change ($\pm$ S.E.M.) calculated by the comparative 2^{− $\Delta\Delta C_t$} method for the control ($n = 8-10$) and agonist-treated ($n = 8-10$) groups. Statistical significance was assessed by Student's *t*-test and is indicated as * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, compared with the control. Changes that were statistically significant are shown in bold.

Gene Name	HPRT1 Gene		GAPDH Gene	
	Fold Change	Statistical Significance	Fold Change	Statistical Significance
5-CT injection				
<i>CYP2C11</i>	1.78 $\downarrow$ (± 0.44)	$P < 0.05$	1.81 $\downarrow$ (± 0.27)	$P < 0.001$
<i>CYP3A1/23</i>	1.96 $\downarrow$ (± 0.56)	$P < 0.05$	1.61 $\downarrow$ (± 0.41)	$P < 0.05$
<i>CYP3A2</i>	1.28 $\downarrow$ (± 1.21)	$P = 0.081$	1.60 $\downarrow$ (± 1.17)	$P = 0.053$
8-OH-DPAT injection				
<i>CYP2C11</i>	1.35 $\downarrow$ (± 0.21)	$P < 0.05$	1.54 $\downarrow$ (± 0.23)	$P < 0.01$
<i>CYP3A1/23</i>	1.25 $\downarrow$ (± 0.15)	$P < 0.05$	1.54 $\downarrow$ (± 0.22)	$P < 0.05$
<i>CYP3A2</i>	0.92 (± 0.067)	$P = 0.39$	1.19 $\downarrow$ (± 0.24)	$P = 0.09$

A 5-CT (5-HT₁ agonist), 30 min **B** 8-OHDPAT (5-HT_{1A} agonist), 30 min

Fig. 4. The effect of a single injection of the 5-HT₁ receptor agonist 5-CT (A) and the 5-HT_{1A} receptor agonist 8-OH-DPAT (B) into the PVN (5 µg/side) on the serum hormones and pituitary SST, CRH, and TRH levels. All values are the mean ± S.E.M. (*n* = 8–10). Statistical significance was assessed by one-way analysis of variance followed by Duncan's test and is indicated as **P* < 0.05 and ****P* < 0.001, compared with the control. The absolute control values for 5-CT and 8-OH-DPAT were 13.5 ± 0.6 and 77.8 ± 12.7 ng/ml; 187.8 ± 11.2 pg/ml; 5.7 ± 0.25, 68.1 ± 2.9, and 2.9 ± 0.2 ng/ml; 11.7 ± 0.7 pg/mg; 0.97 ± 0.058 ng/mg; and 6.3 ± 0.5 pg/mg for serum GH, CRT, ACTH, T₃, T₄, TSH, SST, CRH, and TRH in the pituitary, respectively.

measured in the blood serum, pituitary, or hypothalamus. The level of serum GH, CRT, and thyroid hormones (T₃ and T₄) that directly regulate P450 expression were measured. Simultaneously, the pituitary-produced hormones, ACTH and TSH, were measured in the serum. Moreover, the PVN-produced hormones, the inhibiting hormone SST, and the releasing hormones CRH and TRH, were measured in the pituitary or hypothalamus (Figs. 4 and 5). The measurements were performed 30 minutes after a single injection (a screening test for acute hormonal effects of the agonists) or 3 hours after the repeated injection of the agonist into the PVN, i.e., at the time when the hormonal effects of the 5-HT₁ agonists on P450 expression and activity were translated and investigated biochemically.

The ELISA results revealed that a single injection of the 5-HT₁/5-HT_{1A} receptor agonists 5-CT and 8-OH-DPAT produced a decrease in the serum concentration of GH (to 81% and 69% of the control, respectively) and an increase in the SST level in the pituitary (to 122% and 116% of the control, respectively) at 30 minutes (Fig. 4, A and B). At 3 hours after the 5-day injection of the agonists, these hormonal effects were even far more pronounced in the case of 5-CT (GH decreased to 30%, pituitary SST increased to 190%, and ARC SST increased to 132% of the control) (Fig. 5A), but they were hardly perceived for 8-OH-DPAT (Fig. 5B).

Moreover, the serum CRT concentration significantly decreased to 22% of the control at 30 minutes after a single injection of 8-OH-DPAT

A 5-CT (5-HT₁ agonist), 3h **B** 8-OHDPAT (5-HT_{1A} agonist), 3h

Fig. 5. The effect of the repeated injection of the 5-HT₁ receptor agonist 5-CT (A) and the 5-HT_{1A} receptor agonist 8-OH-DPAT (B) into the PVN (5 µg/side) on the serum, pituitary, and hypothalamic hormone levels. All values are the mean ± S.E.M. (*n* = 8–10). Statistical significance was assessed by Student's *t*-test and is indicated as **P* < 0.05, ***P* < 0.01, and ****P* < 0.001, compared with the control. The absolute control values for 5-CT were 12.45 ± 0.67 and 153.53 ± 21.95 ng/ml; 114.71 ± 19.70 pg/ml; 3.05 ± 0.23, 162 ± 16.85, and 3.57 ± 0.14 ng/ml; 33.90 ± 8.51 pg/mg; 2.59 ± 0.155 ng/mg; and 22.78 ± 0.95, 42.63 ± 3.08, 4.00 ± 0.77, and 6.29 ± 0.68 pg/mg for serum GH, CRT, ACTH, T₃, T₄, TSH, SST, CRH, and TRH in the pituitary; CRH and TRH in the PVN; and SST in the ARC, respectively. The absolute control values for 8-OH-DPAT were 3.76 ± 0.93, 244.21 ± 42.64, 206.87 ± 4.34, 6.31 ± 1.65, 316.18 ± 7.81, and 1.48 ± 0.22 ng/ml; 31.35 ± 1.68 pg/mg; 1.14 ± 0.15 ng/mg; 8.48 ± 0.90 and 128.89 ± 9.96 pg/mg; 4.67 ± 0.86 ng/mg; and 5.76 ± 0.75 pg/mg for serum GH, CRT, ACTH, T₃, T₄, TSH, SST, CRH, and TRH in the pituitary; CRH and TRH in the PVN; and SST in the ARC, respectively.

(Fig. 4B) but not for 5-CT (Fig. 4A). Such a decrease, although less pronounced (57% of the control), was maintained at 3 hours after the 5-day injection of 8-OH-DPAT (Fig. 5B). At the same time, the serum concentration of ACTH and the pituitary level of CRH were not changed (neither at 30 minutes nor at 3 hours), while the hypothalamic level of CRH in the PVN area was enhanced at 3 hours after repeated injection of 5-CT or 8-OH-DPAT (to 121% and 127% of the control, respectively) (Fig. 5B).

In addition, the serum concentration of TSH was augmented at 30 minutes after a single injection of 5-CT or 8-OH-DPAT (to 251% and 205% of the control, respectively), while the level of pituitary TRH and the concentrations of the thyroid hormones T₃ and T₄ were not changed (Fig. 4, A and B). The level of serum TSH returned to the control value in the case of 5-CT but was reduced in the case of 8-OH-DPAT (to 50% of the control) at 3 hours after the 5-day injection of the agonists (Fig. 5, A and B). At the same time, the serum concentration of T₄ was elevated (to 139% of the control), and that of T₃ was diminished (to 69% of the control) after the repeated injection of 5-CT (Fig. 5A). However, no change in thyroid hormone concentrations (T₃ or T₄) was observed after the repeated injection of 8-OH-DPAT (Fig. 5B). However, the TRH levels in the hypothalamic PVN area and pituitary were significantly

Fig. 6. The central neuroendocrine regulation of liver P450 via 5-HT_{1A} receptor located in the PVN (the scheme is based on the results presented in the article). The activation of the 5-HT_{1A} receptor in the PVN of the hypothalamus increases SST secretion and decreases GH and CRT concentration in the blood, which leads to a reduction in hormone-dependent CYP2C11 and CYP3A1/23 expression and activity in the liver.

increased at 3 hours after the repeated injection of 5-CT or 8-OH-DPAT (to 196% and 154% of the control for PVN and to 112% and 127% for the pituitary, respectively). The serum concentrations of IL-2 and IL-6 were not significantly changed by the single or repeated administration of 5-CT or 8-OH-DPAT into the PVN (data not shown).

Discussion

The results of our previous study suggested that the serotonergic innervation of the PVN of the hypothalamus negatively regulates P450 (CYP2C11) via a neuroendocrine mechanism involving GH (Rysz et al., 2016b). Our present study, carried out after injection of type 5-HT₁ and 5-HT₂ serotonergic receptor agonists into the PVN, allowed us to identify the receptor subtype engaged in that regulation. It has been revealed that serotonergic 5-HT_{1A} receptors, but not 5-HT_{1B/D} subtype or 5-HT₂ type receptors, present therein are engaged in the central neuroendocrine regulation of liver P450 by the brain serotonergic system. The regulation of P450 by 5-HT_{1A} receptors of the PVN is negative, which corresponds well with the observed positive effect of PVN lesion on enzyme expression (Rysz et al., 2016b). It is also noteworthy that the effect of 5-HT_{1A} receptors of the PVN on liver P450 was observed after administration of a pharmacologically effective dose of the agonist 8-OH-DPAT into the PVN (5 µg/side). The applied dose was shown to induce a characteristic set of behavioral responses that were blocked by pretreatment with the 5-HT_{1A} receptor antagonist pMPPF given into the PVN (de Souza Villa et al., 2008). The latter finding strongly testifies to the engagement of brain 5-HT_{1A} receptors of the PVN in the regulation of liver P450 expression.

Rats receiving an injection of the nonselective 5-HT₁ receptor-type agonist 5-CT into the PVN for 5 days showed decreased activity of the

principal rat male P450 isoform CYP2C11 in the liver at 3 hours after the last injection. In contrast, the repeated injection of the nonselective 5-HT₂ receptor-type agonist DOI into the PVN did not affect the activity of CYP2C11. Further studies into the 5-HT₁ receptor subtype responsible for the aforementioned effect of 5-CT showed that the 5-day injection of the selective 5-HT_{1A} receptor agonist 8-OH-DPAT into the PVN significantly diminished the activity of CYP2C11 at 3 hours after the last injection, while the repeated injection of the 5-HT_{1B/D} receptor agonist sumatriptan did not affect the CYP2C11 activity. The aforementioned results show that the 5-HT_{1A} serotonergic receptor subtype of PVN is engaged in the negative regulation of the activity of liver CYP2C11. The observed increase in the CYP2A activity after 5-CT injection does not seem to be connected with 5-HT_{1A} or 5-HT_{1B/D} receptor activation since such a change does not appear after the administration of 8-OH-DPAT or sumatriptan, but may stem from the affinity of 5-CT for other 5-HT receptors present in the hypothalamus (Hoyer et al., 1994; Wesołowska, 2002).

The decreased activity of CYP2C11, produced by the repeated injection of the 5-HT₁ receptor agonist 5-CT or the 5-HT_{1A} receptor agonist 8-OH-DPAT into the PVN, correlated positively with the enzyme expression level (mRNA and protein) in the liver. This indicates that the activation of 5-HT_{1A} serotonergic receptor subtype in the PVN inhibits the synthesis of the CYP2C11 protein in the liver at the transcriptional level, the effect being opposite to that observed previously after the lesion of the serotonergic innervation of the PVN (Rysz et al., 2016b). Moreover, the CYP3A1/23 expression (mRNA and protein levels) also decreased, which was consistent with the observed tendency to decrease the CYP3A activity measured as the rate of 6β-hydroxylation of testosterone. The reaction is characteristic for both CYP3A1/23 and CYP3A2 isoforms, the first one being expressed constitutively to a lesser degree (Gibson et al., 2002).

Liver P450 is regulated hormonally by pituitary-produced GH, adrenal CRT, and thyroid hormones (T_3 and T_4). Growth hormone is a chief factor stimulating the expression of the rat male *CYP2C11* gene as a result of its pulsatile secretion by the anterior pituitary gland (Waxman et al., 1995; Waxman and O'Connor, 2006; Waxman and Holloway, 2009). Corticosterone can also regulate the *CYP2C11* isoform. At its lower concentration CRT stimulates, and at its higher (stress) concentration it inhibits, the expression of *CYP2C11* by increasing the level of hypothalamic SST and thus inhibiting GH release (Strobl and Thomas, 1994; Devesa et al., 1995; Iber et al., 1997; Mazziotti and Giustina, 2013). However, CRT is foremost one of the main regulators of *CYP3A* subfamily isoforms (Gibson et al., 2002). On the other hand, thyroid hormones are known to inhibit the expression of different P450 isoforms (Yamazoe et al., 1989; Murayama et al., 1991; Liddle et al., 1998). In our experiment, the 5-HT_{1A}/5-HT_{1A} receptor agonist-evoked decreases in P450 activity and expression were accompanied by changes in the serum concentrations of GH, CRT, and thyroid hormones, as well as in the levels of pituitary-produced TSH and the PVN-produced inhibiting hormones SST, CRH, and TRH.

Thus, both the 5-HT₁ receptor agonist 5-CT and the 5-HT_{1A} receptor agonist 8-OH-DPAT produced a significant decrease in the serum concentration of GH simultaneously with the increase in the level of pituitary SST at 30 minutes and/or 3 hours after a single or repeated injection into the PVN (a screening test for acute hormonal effects of the agonists). The observed opposite changes in the concentrations of the two hormones are physiologically consistent since SST is known to inhibit the secretion of GH at the level of the pituitary gland and ARC nucleus (Liposits et al., 1988; Tannenbaum, 1991; Fodor et al., 1994; Bluet-Pajot et al., 1998; McMahon et al., 2001). It can be suggested that the activation of 5-HT_{1A} presynaptic receptors on GABA neurons inhibits the release of the major inhibitory neurotransmitter GABA and GABA_A receptor-mediated signaling in SST neurons, exerting its stimulatory effect on SST secretion (Rage et al., 1992, 1994; Herbison and Augood, 1994; Murray et al., 1999; Lee et al., 2008), and in turn leading to inhibitory effects on GH secretion and GH-dependent P450 expression. The observed effects agree with the results of some pharmacological experiments showing that the intravenous administration of 8-OH-DPAT to rats decreased plasma GH concentration (Aulakh et al., 1988). Moreover, 8-OH-DPAT significantly decreased the serum CRT concentration at 30 minutes and 3 hours after a single or repeated injection. At 3 hours after the repeated administration of the agonists, which is the time when the hormonal effects of the 5-HT₁ agonists affected P450 expression and activity, these hormonal effects were even more pronounced in the case of 5-CT (GH and SST) but were attenuated in the case of 8-OH-DPAT (GH, SST, and CRT). The latter effects may be due to a faster elimination rate of 8-OH-DPAT than 5-CT from the PVN and/or desensitization of the 5-HT_{1A} receptor produced by the repeated injection of the selective agonist 8-OH-DPAT (Raap and Van de Kar, 1999). However, both 30-minute and 3-hour hormonal responses may contribute to the effect of 8-OH-DPAT on P450 expression observed at 3 hours after injection. Therefore, it seems that the diminished expression of *CYP2C11* and *CYP3A1/23* was produced by the decrease in the secretion of GH and CRT since these hormonal effects were observed at 30 minutes and/or 3 hours after single or repeated 5-HT_{1A} receptor activation in the PVN. The observed decrease in the CRT level after the selective activation of the 5-HT_{1A} receptor by 8-OH-DPAT could be masked in the case of the nonselective activation of 5-HT₁ receptors by 5-CT because of the simultaneous stimulation of the 5-HT_{1A} receptor and other 5-HT receptor subtypes (Hoyer et al., 1994; Wesolowska, 2002).

Some dynamic, time-dependent changes in the function of the hypothalamic-pituitary-thyroidal axis observed after the injection of

5-CT or 8-OH-DPAT into the PVN (i.e., changes in the TRH, TSH, or T_3 and T_4 levels) might also have certain consequences for P450. Changes in the serum concentration of thyroid hormones (T_4 elevation and T_3 reduction) after repeated 5-CT injection might attenuate the effect of the hypothalamic-pituitary-thyroidal axis on *CYP3A* expression since T_3 , which negatively regulates *CYP3A*, is a much stronger agonist for the thyroid receptor than T_4 (Yamazoe et al., 1989; Liddle et al., 1998). However, the observed changes in the serum concentration of thyroid hormones may not be connected with the activation of the 5-HT_{1A} subtype receptor since they do not occur after the injection of the selective 5-HT_{1A} receptor agonist 8-OH-DPAT. The serum concentrations of IL-2 and IL-6, which negatively regulate P450 expression, were not changed in our experiment, which justifies the proposed neuroendocrine interpretation of the obtained results.

In conclusion, the intracerebral injection of different specific 5-HT₁ and 5-HT₂ receptor agonists at pharmacologically active doses into the hypothalamic PVN in vivo, followed by the measurement of hypothalamic, pituitary, and peripheral hormone levels and liver P450 expression, revealed a new brain serotonergic mechanism engaged in the neuroendocrine regulation of P450. The obtained results indicate that the serotonergic 5-HT_{1A} receptors, but not the 5-HT_{1B/D} subtype or 5-HT₂ type receptors present in the PVN, are involved in the central neuroendocrine regulation of P450 in the liver. The activation of the 5-HT_{1A} receptor in the PVN produces an increase in SST secretion and a decrease in GH and CRT concentration in the blood, which leads to a reduction in hormone-dependent *CYP2C11* and *CYP3A1/23* expression and activity in the liver (Fig. 6). Since 5-HT_{1A} receptors are involved in the pharmacological action of antidepressant, antianxiety, and neuroleptic drugs, treatment with these psychotropics may negatively regulate hormone-dependent P450 isoforms in the liver via brain neuroendocrine mechanisms.

Authorship Contributions

Participated in research design: Daniel.

Conducted experiments: Bromek, Rysz, Haduch, Wójcikowski.

Performed data analysis: Bromek, Rysz, Haduch, Daniel.

Wrote or contributed to the writing of the manuscript: Bromek, Rysz, Daniel.

References

- Aulakh CS, Wozniak KM, Haas M, Hill JL, Zohar J, and Murphy DL (1988) Food intake, neuroendocrine and temperature effects of 8-OHDPAT in the rat. *Eur J Pharmacol* **146**: 253–259.
- Bluet-Pajot MT, Epelbaum J, Gourdi D, Hammond C, and Kordon C (1998) Hypothalamic and hypophyseal regulation of growth hormone secretion. *Cell Mol Neurobiol* **18**:101–123.
- Bromek E, Wójcikowski J, and Daniel WA (2013) Involvement of the paraventricular (PVN) and arcuate (ARC) nuclei of the hypothalamus in the central noradrenergic regulation of liver cytochrome P450. *Biochem Pharmacol* **86**:1614–1620.
- Brtko J and Dvorak Z (2011) Role of retinoids, retinoids and thyroid hormone in the expression of cytochrome P450 enzymes. *Curr Drug Metab* **12**:71–88.
- Clissold KA, Choi E, and Pratt WE (2013) Serotonin 1A, 1B, and 7 receptors of the rat medial nucleus accumbens differentially regulate feeding, water intake, and locomotor activity. *Pharmacol Biochem Behav* **112**:96–103.
- Currie PJ, John CS, Nicholson ML, Chapman CD, and Loera KE (2010) Hypothalamic paraventricular 5-hydroxytryptamine inhibits the effects of ghrelin on eating and energy substrate utilization. *Pharmacol Biochem Behav* **97**:152–155.
- de Paula DC, Torricelli AS, Lopreato MR, Nascimento JO, and Viana MB (2012) 5-HT_{2A} receptor activation in the dorsolateral septum facilitates inhibitory avoidance in the elevated T-maze. *Behav Brain Res* **226**:50–55.
- de Souza Villa P, Menani JV, de Arruda Camargo GM, de Arruda Camargo LA, and Saad WA (2008) Activation of the serotonergic 5-HT_{1A} receptor in the paraventricular nucleus of the hypothalamus inhibits water intake and increases urinary excretion in water-deprived rats. *Regul Pept* **150**:14–20.
- Devesa J, Barros MG, Gondar M, Tresguerres JA, and Arce V (1995) Regulation of hypothalamic somatostatin by glucocorticoids. *J Steroid Biochem Mol Biol* **53**:277–282.
- Dvorak Z and Pavek P (2010) Regulation of drug-metabolizing cytochrome P450 enzymes by glucocorticoids. *Drug Metab Rev* **42**:621–635.
- Fletcher PJ, Azampanah A, and Korth KM (2002) Activation of 5-HT_{1B} receptors in the nucleus accumbens reduces self-administration of amphetamine on a progressive ratio schedule. *Pharmacol Biochem Behav* **71**:717–725.
- Fletcher PJ and Korth KM (1999) Activation of 5-HT_{1B} receptors in the nucleus accumbens reduces amphetamine-induced enhancement of responding for conditioned reward. *Psychopharmacology (Berl)* **142**:165–174.

- Fodor M, Csaba Z, Kordon C, and Epelbaum J (1994) Growth hormone-releasing hormone, somatostatin, galanin and β -endorphin afferents to the hypothalamic periventricular nucleus. *J Chem Neuroanat* **8**:61–73.
- Gibson GG, Plant NJ, Swales KE, Ayrton A, and El-Sankary W (2002) Receptor-dependent transcriptional activation of cytochrome P4503A genes: induction mechanisms, species differences and interindividual variation in man. *Xenobiotica* **32**:165–206.
- Gruber K, McRae-Deguerce A, Wilkin LD, Mitchell LD, and Johnson AK (1987) Forebrain and brainstem afferents to the arcuate nucleus in the rat: potential pathways for the modulation of hypothalamic secretions. *Neurosci Lett* **75**:1–5.
- Guo JD and Rannin DG (2010) Presynaptic 5-HT_{1B} receptor-mediated serotonergic inhibition of glutamate transmission in the bed nucleus of the stria terminalis. *Neuroscience* **165**:1390–1401.
- Haduch A, Wójcikowski J, and Daniel WA (2006) The effect of tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs) and newer antidepressant drugs on the activity and level of rat CYP3A. *Eur Neuropsychopharmacol* **16**:178–186.
- Haduch A, Wójcikowski J, and Daniel WA (2008) Effect of selected antidepressant drugs on cytochrome P450 2B (CYP2B) in rat liver. An in vitro and in vivo study. *Pharmacol Rep* **60**: 957–965.
- Herbison AE and Augood SJ (1994) Expression of GABA_A receptor α_2 sub-unit mRNA by periventricular somatostatin neurones in the rat hypothalamus. *Neurosci Lett* **173**:9–13.
- Hopwood SE and Stamford JA (2001) Multiple 5-HT₁ autoreceptor subtypes govern serotonin release in dorsal and median raphe nuclei. *Neuropharmacology* **40**:508–519.
- Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, and Humphrey PP (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol Rev* **46**:157–203.
- Iber H, Chen Q, Sewer M, and Morgan ET (1997) Regulation of hepatic cytochrome P450 2C11 by glucocorticoids. *Arch Biochem Biophys* **345**:305–310.
- Johnson DE, Rollemma H, Schmidt AW, and McHarg AD (2001) Serotonergic effects and extra-cellular brain levels of eletriptan, zolmitriptan and sumatriptan in rat brain. *Eur J Pharmacol* **425**: 203–210.
- Jørgensen HS (2007) Studies on the neuroendocrine role of serotonin. *Dan Med Bull* **54**:266–288.
- Katz LM, Nathan L, Kuhn CM, and Schanberg SM (1996) Inhibition of GH in maternal separation may be mediated through altered serotonergic activity at 5-HT_{2A} and 5-HT_{2C} receptors. *Psychoneuroendocrinology* **21**:219–235.
- Kot M and Daniel WA (2008) Relative contribution of rat cytochrome P450 isoforms to the metabolism of caffeine: the pathway and concentration dependence. *Biochem Pharmacol* **75**: 1538–1549.
- Kot M and Daniel WA (2011) Cytochrome P450 is regulated by noradrenergic and serotonergic systems. *Pharmacol Res* **64**:371–380.
- Kot M, Sadakierska-Chudy A, Haduch A, Rysz M, Bromek E, Golembiowska K, and Daniel WA (2015) The role of the dorsal noradrenergic pathway of the brain (locus coeruleus) in the regulation of liver cytochrome P450 activity. *Eur J Pharmacol* **751**:34–41.
- Larsen PJ, Hay-Schmidt A, Vrang N, and Mikkelsen JD (1996) Origin of projections from the midbrain raphe nuclei to the hypothalamic paraventricular nucleus in the rat: a combined retrograde and anterograde tracing study. *Neuroscience* **70**:963–988.
- Lee KS, Han TH, Jo JY, Kang G, Lee SY, Ryu PD, Im JH, Jeon BH, and Park JB (2008) Serotonin inhibits GABA synaptic transmission in presympathetic paraventricular nucleus neurons. *Neurosci Lett* **439**:138–142.
- Liddle C, Goodwin BJ, George J, Tapner M, and Farrell GC (1998) Separate and interactive regulation of cytochrome P450 3A4 by triiodothyronine, dexamethasone, and growth hormone in cultured hepatocytes. *J Clin Endocrinol Metab* **83**:2411–2416.
- Liposits Z, Merchenthaler I, Paull WK, and Flerkó B (1988) Synaptic communication between somatostatinergic axons and growth hormone-releasing factor (GRF) synthesizing neurons in the arcuate nucleus of the rat. *Histochemistry* **89**:247–252.
- Mamede Rosa ML and Prado WA (1997) Antinociception induced by opioid or 5-HT agonists microinjected into the anterior preoptic nucleus of the rat. *Brain Res* **757**:133–138.
- Mazzioti G and Giustina A (2013) Glucocorticoids and the regulation of growth hormone secretion. *Nat Rev Endocrinol* **9**:265–276.
- McMahon CD, Radcliff RP, Lookingland KJ, and Tucker HA (2001) Neuroregulation of growth hormone secretion in domestic animals. *Domest Anim Endocrinol* **20**:65–87.
- Monostory K and Dvorak Z (2011) Steroid regulation of drug-metabolizing cytochromes P450. *Curr Drug Metab* **12**:154–172.
- Monostory K, Pascucci JM, Kóbori L, and Dvorak Z (2009) Hormonal regulation of CYP1A expression. *Drug Metab Rev* **41**:547–572.
- Mota A, Bento A, Peñalva A, Pombo M, and Dieguez C (1995) Role of the serotonin receptor subtype 5-HT_{1D} on basal and stimulated growth hormone secretion. *J Clin Endocrinol Metab* **80**:1973–1977.
- Murakami Y, Kato Y, Kabayama Y, Tojo K, Inoue T, and Imura H (1986) Involvement of growth hormone (GH)-releasing factor in GH secretion induced by serotonergic mechanisms in conscious rats. *Endocrinology* **119**:1089–1092.
- Murayama N, Shimada M, Yamazoe Y, and Kato R (1991) Difference in the susceptibility of two phenobarbital-inducible forms, P450IIB1 and P450IIB2, to thyroid hormone- and growth hormone-induced suppression in rat liver: phenobarbital-inducible P450IIB2 suppression by thyroid hormone acting directly, but not through the pituitary system. *Mol Pharmacol* **39**:811–817.
- Murray HE, Rantle CM, Simonian SX, DonCarlos LL, Herbison AE, and Gillies GE (1999) Sexually dimorphic ontogeny of GABAergic influences on periventricular somatostatin neurons. *Neuroendocrinology* **70**:384–391.
- National Institute of Health, National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals (2011) *Guide for the care and use of laboratory animals*, 8th ed, National Academies Press, Washington, DC.
- Paxinos G and Watson C (2007) *The Rat Brain in Stereotaxic Coordinates*, 6th ed. Academic Press, London.
- Piñeyro G and Blier P (1996) Regulation of 5-hydroxytryptamine release from rat midbrain raphe nuclei by 5-hydroxytryptamine_{1D} receptors: effect of tetrodotoxin, G protein inactivation and long-term antidepressant administration. *J Pharmacol Exp Ther* **276**:697–707.
- Pitchot W, Wauthy J, Legros JJ, and Anseau M (2004) Hormonal and temperature responses to fentanyl in normal volunteers: an antagonist study. *Eur Neuropsychopharmacol* **14**:151–155.
- Raap DK and Van de Kar LD (1999) Selective serotonin reuptake inhibitors and neuroendocrine function. *Life Sci* **65**:1217–1235.
- Rage F, Benyassi A, Arancibia S, and Tapia-Arancibia L (1992) Gamma-aminobutyric acid-glutamate interaction in the control of somatostatin release from hypothalamic neurons in primary culture: in vivo corroboration. *Endocrinology* **130**:1056–1062.
- Rage F, Rougeot C, and Tapia-Arancibia L (1994) GABA_A and NMDA receptor activation controls somatostatin messenger RNA expression in primary cultures of hypothalamic neurons. *Neuroendocrinology* **60**:470–476.
- Rysz M, Bromek E, and Daniel WA (2016a) Activation of brain serotonergic system by repeated intracerebral administration of 5-hydroxytryptophan (5-HTP) decreases the expression and activity of liver cytochrome P450. *Biochem Pharmacol* **99**:113–122.
- Rysz M, Bromek E, Haduch A, Liskova B, Wójcikowski J, and Daniel WA (2016b) The reverse role of the hypothalamic paraventricular (PVN) and arcuate (ARC) nuclei in the central serotonergic regulation of the liver cytochrome P450 isoform CYP2C11. *Biochem Pharmacol* **112**:82–89.
- Rysz M, Bromek E, Haduch A, Sadakierska-Chudy A, and Daniel WA (2015) Damage to the brain serotonergic system increases the expression of liver cytochrome P450. *Drug Metab Dispos* **43**: 1345–1352.
- Sadakierska-Chudy A, Haduch A, Rysz M, Golembiowska K, and Daniel WA (2013) The role of brain noradrenergic system in the regulation of liver cytochrome P450 expression. *Biochem Pharmacol* **86**:800–807.
- Sawchenko PE, Swanson LW, Steinbusch HWM, and Verhofstad AAJ (1983) The distribution and cells of origin of serotonergic inputs to the paraventricular and supraoptic nuclei of the rat. *Brain Res* **277**:355–360.
- Seletti B, Benkelfat C, Blier P, Annable L, Gilbert F, and de Montigny C (1995) Serotonin_{1A} receptor activation by flesinoxan in humans. Body temperature and neuroendocrine responses. *Neuropsychopharmacology* **13**:93–104.
- Strobl JS and Thomas MJ (1994) Human growth hormone. *Pharmacol Rev* **46**:1–34.
- Tannenbaum GS (1991) Neuroendocrine control of growth hormone secretion. *Acta Paediatr Scand Suppl* **372**:5–16.
- Toivonen M, Rauhala P, and Männistö PT (1990) Site-dependent action of intracerebroventricular 5-hydroxytryptamine on the cold-stimulated thyrotropin secretion in male rats. *Neuroendocrinology* **51**:45–50.
- Tuomisto J and Männistö P (1985) Neurotransmitter regulation of anterior pituitary hormones. *Pharmacol Rev* **37**:249–332.
- Valverde I, Penalva A, and Dieguez C (2000) Influence of different serotonin receptor subtypes on growth hormone secretion. *Neuroendocrinology* **71**:145–153.
- Waxman DJ and Holloway MG (2009) Sex differences in the expression of hepatic drug metabolizing enzymes. *Mol Pharmacol* **76**:215–228.
- Waxman DJ and O'Connor C (2006) Growth hormone regulation of sex-dependent liver gene expression. *Mol Endocrinol* **20**:2613–2629.
- Waxman DJ, Ram PA, Pampori NA, and Shapiro BH (1995) Growth hormone regulation of male-specific rat liver P450s 2A2 and 3A2: induction by intermittent growth hormone pulses in male but not female rats rendered growth hormone deficient by neonatal monosodium glutamate. *Mol Pharmacol* **48**:790–797.
- Wesolowska A (2002) In the search for selective ligands of 5-HT₅, 5-HT₆ and 5-HT₇ serotonin receptors. *Pol J Pharmacol* **54**:327–341.
- Willins DL and Meltzer HY (1997) Direct injection of 5-HT_{2A} receptor agonists into the medial prefrontal cortex produces a head-twitch response in rats. *J Pharmacol Exp Ther* **282**:699–706.
- Willoughby JO and Blessing WW (1987) Origin of serotonin innervation of the arcuate and ventromedial hypothalamic region. *Brain Res* **418**:170–173.
- Willoughby JO, Menadeu MF, and Liebelt H (1987) Activation of serotonin receptors in the medial basal hypothalamus stimulates growth hormone secretion in the unanesthetized rat. *Brain Res* **404**:319–322.
- Wójcikowski J and Daniel WA (2009) The brain dopaminergic system as an important center regulating liver cytochrome P450 in the rat. *Expert Opin Drug Metab Toxicol* **5**:631–645.
- Wójcikowski J and Daniel WA (2011) The role of the nervous system in the regulation of liver cytochrome P450. *Curr Drug Metab* **12**:124–138.
- Wójcikowski J, Golembiowska K, and Daniel WA (2007) The regulation of liver cytochrome P450 by the brain dopaminergic system. *Curr Drug Metab* **8**:631–638.
- Wójcikowski J, Golembiowska K, and Daniel WA (2008) Regulation of liver cytochrome P450 by activation of brain dopaminergic system: physiological and pharmacological implications. *Biochem Pharmacol* **76**:258–267.
- Wójcikowski J, Haduch A, and Daniel WA (2013) Effect of antidepressant drugs on cytochrome P450 2C11 (CYP2C11) in rat liver. *Pharmacol Rep* **65**:1247–1255.
- Yamazoe Y, Murayama N, Shimada M, and Kato R (1989) Thyroid hormone suppression of hepatic levels of phenobarbital-inducible P-450b and P-450c and other neonatal P-450s in hypophysectomized rats. *Biochem Biophys Res Commun* **160**:609–614.
- Zhang Y, Gray TS, D'Souza DN, Carrasco GA, Damjanoska KJ, Dudas B, Garcia F, Zainelli GM, Sullivan Hanley NR, Battaglia G, et al. (2004) Desensitization of 5-HT_{1A} receptors by 5-HT_{2A} receptors in neuroendocrine neurons in vivo. *J Pharmacol Exp Ther* **310**:59–66.

Address correspondence to: Władysława A. Daniel, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, 31-343 Kraków, Poland. E-mail: nfdaniel@cyf-kr.edu.pl